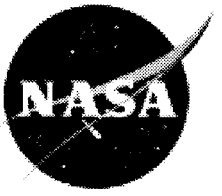

Mars Sample Handling Protocol Workshop Series

Interim Report: Workshop 1
Bethesda, Maryland
March 20-22, 2000

Edited by:

Margaret S. Race
SETI Institute

John D. Rummel
NASA, Washington, D.C.

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(October 2000)

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PREFACE

Numerous NASA reports and studies have identified Planetary Protection (PP) as an important part of a Mars Sample Return mission. The mission architecture, hardware, and activities must be designed in ways that prevent both forward- and back-contamination, and ensure maximal return of scientific information. A key element of planetary protection for sample return missions is the development of guidelines for returned sample containment and 'biomarker' analysis.

In 1997, a Mars Sample Quarantine Protocol workshop *[DeVincenzi et al. 1999]* was convened at NASA Ames Research Center to deal with three specific aspects of the initial handling of a returned Mars sample: 1) biocontainment, to prevent 'uncontrolled release' of sample material into the terrestrial environment; 2) life detection, to examine the sample for evidence of organisms; and 3) biohazard testing, to determine if the sample poses any threat to terrestrial life forms and the Earth's biosphere. In 1999, a study by NASA's Mars Sample Handling and Requirements Panel (MSHARP) *[Carr, et al. 1999]* addressed three other specific areas in anticipation of returning samples from Mars: 1) sample collection and transport back to Earth; 2) certification of the samples as non-hazardous; and 3) sample receiving, curation, and distribution.

To further refine the requirements for sample hazard testing and the criteria for subsequent release of sample materials from quarantine, the NASA Planetary Protection Officer convened an additional series of workshops beginning in March 2000. The overall objective of these workshops is to develop comprehensive protocols to assess whether the returned materials contain any biological hazards, and to safeguard the purity of the samples from possible terrestrial contamination. This document is the report of the first workshop in this additional workshop series. The information herein will ultimately be integrated into a final document from the entire workshop series along with additional information and recommendations (see pages 9 and 13 for further comment).

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EXECUTIVE SUMMARY

In anticipation of a Mars sample return mission sometime in the next decade, it will be necessary to prepare for handling and testing of martian materials here on the Earth. Previous groups and committees have studied selected aspects of sample return activities, but specific detailed protocols for handling and testing must still be developed. To further refine the requirements for sample hazard testing and to develop the criteria for subsequent release of sample materials from quarantine, the NASA Planetary Protection Officer convened a series of workshops beginning in 2000. The overall objective of the workshop series is to develop comprehensive draft protocols by which returned martian sample materials could be assessed for biological hazards and to safeguard the purity of the samples from possible terrestrial contaminants.

This document is the report resulting from the first workshop of the series, which was held in Bethesda, Maryland on March 20-22, 2000. This report serves to document the proceedings of Workshop 1; it summarizes relevant background information, provides an overview of the deliberations to date, and helps frame issues that will need further attention or resolution in upcoming workshops. Specific recommendations are not part of this report.

Individual Sub-groups were created during Workshop 1 to discuss specific assigned topics. The views and findings expressed by these Sub-groups are preliminary in nature and are not intended to represent a consensus of all participants of Workshop 1. Furthermore, the findings reported herein may not be consistent with the final report and recommendations to be issued at the conclusion of the entire workshop series. Although the goal of developing an actual sample-handling protocol is still a long way off, there are areas of consensus emerging, which will be helpful towards that end. To date, the preliminary deliberations and findings of the Sub-groups from Workshop 1 are summarized here (the complete Sub-group reports are included in this document beginning on page 17).[1]

Sub-group 1: Preliminary Sample Characterization Requirements

Sub-group 1 identified specific data and information that should be collected or recorded about the samples in order to facilitate maximum scientific information. This Sub-group specified that the data should include: information related to the collection site itself, physical characteristics of each specimen, microscopic examination and cross-sections, elemental abundances, mineralogical characterization, non-destructive evaluation of cracks and defects in rock samples, surface reactivity and chemistry, and evaluation of total and organic carbon. In addition, Sub-group 1 highlighted the critical need for further discussions on questions about sterilization of sub-samples[2] prior to their distribution.

1. During the Workshop, all participants were divided into Sub-groups based on their background and area(s) of expertise and the assigned topics to be discussed. On Day 1, the Sub-groups met for approximately 2 hours. On Day 2, participants were divided into 3 new Sub-groups which met for day-long, in-depth discussions; these same Sub-groups also met on the morning of Day 3 before reporting a summary of their deliberations to the entire Workshop in a final Plenary session.
2. According to the Space Studies Board (SSB), Task Group on Issues in Sample Return, Mars Sample Return: Issues and Recommendations, National Academy Press, Washington, D.C. (1997), " ... if any portion of the sample is removed (from containment) prior to completion of ... analyses, it should first be sterilized." (p. 4). To date, no decisions have been made about sterilization of sub-samples, including the method(s) to be used. At this time, plans are underway to organize a separate Workshop specifically to address questions and issues about sterilization of returned martian sample materials.

Combined Sub-groups 2 and 4: Sub-group 2: Representative Sub-samples; Nature of Sample; Sub-group 4: Physical/Chemical Analyses; Methods, Sample State, Containment, and Controls

Although Sub-groups 2 and 4 met separately and were assigned two different discussion topics, they decided to prepare a joint report. Because of their areas of expertise, the members of these two Sub-groups overlapped to a great degree; moreover, the discussions complemented each other because of the focus on the nature and characterization of incoming samples. For the purpose of their combined written summary, they retroactively revised their separate charters to read as one combined charter, as follows:

> *"Establish a protocol for documenting, sub-dividing, and characterizing the samples; specifying the nature and sequence of physical, chemical, and mineralogic tests necessary to support the tasks of life detection, biohazard analysis, and preliminary examination for the benefit of the scientific user community."*

The combined Sub-group also proposed a set of operating principles, which they recommend be applied to all activities within the Sample Receiving Facility (SRF). These principles, which represent a concise statement of issues discussed during their sessions (particularly during the discussions by Sub-group 4), include recommendations that all tests be done with the absolute minimum amount of sample necessary; that handling, testing, and characterization activities do the least harm to the returned martian materials; and that geochemical and mineralogic analyses be kept to the minimum necessary to support the protocol.

Sub-groups 2 and 4 constructed a proposed protocol flow chart (see figure 1, page 22) for sample characterization and subdivision, dividing the process into five separate steps that dealt with all three categories of samples (e.g., atmosphere, fines, and rocks). The steps in their process include:

1. Sample Removal and Basic Documentation: extracting and filtering the gas; opening the sample container, removing the sample, and recording basic physical, photographic and curatorial information.
2. Preliminary Characterization: selection of representative sample materials for testing purposes via preliminary visual and gross geological/mineralogical examination, followed by selected non-destructive and non-invasive methods to characterize individual samples; and finally, some fraction of materials selected for testing, while a remaining fraction is stored for future scientific research.
3. Splitting: separating sample types by size fractions or other criteria for use in current protocol testing and/or future scientific testing; sample types distinguished as fines, pebbles, rock cores, and complex pebbles/rocks.
4. Detailed Examination and Analysis (physical chemistry and mineralogy only): analyses to include bulk chemistry, mineralogy, total carbon, preliminary organic carbon analyses, total water assay, and petrography.
5. Release from Containment: samples will either be sterilized or released from containment for controlled distribution, depending upon results from protocol tests.

Any mention of sterilization in this document is based on an acknowledgement that some sub-samples of martian materials may be sterilized and released from containment to perform tests that are part of the overall protocol.

Sub-group 3: Sequence of Tests; Types of Testing Possible; Range of Results re: Release Criteria

This Sub-group was charged with addressing the end-to-end requirements of an effective sample-testing protocol, using the strawman protocol as a point of departure. Nonetheless, the write-up from Sub-group 3 focused primarily on biohazard assessment, biohazard clearance (i.e., determination of the absence of any biohazard), and the criteria upon which martian samples could be released to the scientific community.

Sub-group 3 reported four particular constraints and working assumptions to be applied to their sample-handling protocol as developed during their deliberations. These were:

1. Any genuine martian life form if found should be kept under continued containment whether it is hazardous or not;
2. Toxicity should be tested, but it is not a criterion for release;
3 Life detection and biohazard testing partially overlap; and
4. Biohazard testing should explicitly emphasize analytic probes that can identify agents that might live, replicate, or otherwise interact with terrestrial carbon-based systems.

The Sub-group specified four levels of questions and methodological approaches that should guide the biohazard testing process, leading to decisions about whether to release materials from containment. These levels included the sequential search for structural indications of life forms, chemical signatures of life,. evidence of replication, and monitoring for adverse effects on personnel and the environment at the receiving facility.

Finally, Sub-group 3 highlighted four areas needing further attention:

1. Additional input from other government agencies with experience in biohazard testing;
2. Deliberations on what selection of cell and whole organism types should be used in biohazard assessment;
3. Involvement of statistical experts in assessing the validity of sampling and testing plans;
4. Research and consulting on development of micro-scale model systems for assessing potential impacts on ecosystems.

Sub-group 5: Candidate Life Detection Tests- Qualifiers, Contraindications, Controls, and Characterization

Sub-group 5 focused on preliminary identification measurements and tests that should be performed to look for evidence of life or life-related molecules. This Sub-group outlined a series of procedures that will minimally be required to assess for the presence of non-terrestrial life forms in returned martian samples (rocks, soils, and fines). This proposed scheme included initial processing in a nitrogen gas environment at 15°C under strict biocontainment. The Sub-group devised a flow chart (see figure 2, page 27) that suggests sequential processing of various sample types using filtration, fluorescent activated flow cytometry, laser Raman mass spectroscopy, Limulus Amebocyte Lysate (LAL) assays, polymerase chain reaction (PCR) sequencing, micro-scale culturing, broad band fluorescence, and 3-dimensional tomography in a synchrotron. Other analyses that were proposed included tests for chirality and a combination

of capillary electrophoresis, stains, and fluorimetry. Finally, Sub-group 5 suggested that if a survey of samples reveals the absence of carbon or complex organics, the samples can and should be released from the containment facility. If there are indications of biological molecules, more extended testing would, of course, be required.[3]

Sub-group 6: Candidate Biohazard Tests: Qualifiers, Contraindications, Controls, and Characterization

Sub-group 6 sought to determine the preliminary identification of measurements and tests that should be applied to the sample to analyze for biohazards, without regard to evidence of life or life-related molecules within the samples. Sub-group 6 suggested the need for preliminary testing to gather baseline information on the various sample types, including descriptive and physical characteristics, comparative gas analyses, and X-ray imaging and 3-dimensional image analysis using a synchrotron for carbon analyses. Subsequent to the preliminary data collection, the group proposed a stepwise process to be implemented for biohazard analysis using *in vitro* and *in vivo* testing protocols (see figure 3, page 31).

For *in vitro* testing, the group suggested employing primary and established cell lines derived from plants, animals, insects, humans, bacterial and uni-cellular eucaryotic cell cultures (see Sub-group 6 report, page 29 for further details), and if available, microbial community ecosystem models. Tests for possible biohazards should focus on detecting replicative properties of the hazardous entity, selected phenotypic responses, and host-gene expression responses. For *in vivo* testing, the Sub-group suggested using varied model systems including mouse (e.g., knockout mice with immune defects and Specific Pathogen Free (SPF) out-bred mice), plants (e.g., *Arabidopsis* and others), as well as insect and ecosystem models (details TBD). The group also developed two separate decision trees outlining alternative procedural approaches for the biohazard analysis process (see figures 4 and 5, pages 34 and 35).

Upon completion of the *in vitro, in vivo,* and model ecosystem testing, the Sub-group agreed that sample(s) may be selected for release from maximum containment if no biohazard or life form has been detected. The Sub-group suggested, however, that additional experiments and life detection tests be done under level 3 biocontainment subject to case-by-case peer review by an appropriate evaluation panel. Finally, if sub-samples are to be released prior to completion of the protocol testing, the Sub-group stated that the sub-samples should be subjected to extensive gamma irradiation sterilization (dose and time TBD).[4] The group noted that considerable research will have to be done to determine the efficacy of various sterilization methods.[5]

3. To date, no decisions have been made about when and under what conditions sample materials will be eligible for or will actually be released from containment at the Sample Return Facility (SRF). Such decisions will be discussed in later Workshops and will invariably involve considerations of sample sterilization and interpretation of protocol test results. Ultimately, it is likely that decisions about what is done with sample materials will be made after review by an appropriate international scientific oversight committee at the SRF in consultation with NASA's Planetary Protection Officer and other responsible officials.
4. To date, no decisions have been made about sterilization of sub-samples, including the method(s) to be used.
5. At this time, plans are underway to organize a separate Workshop specifically to address questions and issues about sterilization of returned martian sample materials.

Notes

This document is the final report of Workshop 1, but only an interim report of the Workshop series. This report is intended to provide a summary of Workshop 1 to serve as background information for participants of future workshops in the series and any other interested parties. It will also serve as a starting point for deliberations during Workshop 2 (see page 13 for further comments on this topic). *If any portion of this report is to be cited or referenced it must be with the understanding that this document is neither authoritative nor indicative of any final decisions or plans for future Mars missions.*

This Executive Summary was drafted from summaries written by each Sub-group following Workshop 1. The complete summaries, which appear in the main body of this report, have undergone minimal editing. No attempt has been made to reconcile differences between the Sub-groups, nor to determine at this time whether particular suggestions would be feasible or recommended for a Mars sample return mission. Throughout this report, the reader is referred to 'notes' which serve to qualify or clarify the temporary nature of particular statements; these notes appear in Appendix G. The collective thoughts and suggestions of all the Sub-groups will be subject to further discussion at future workshops. The information herein will eventually be integrated with additional findings and recommendations from the entire Workshop series. Upon completion of the Workshop series, a final report for the series will be published.